JP09285298 TAKAYUKI et al

Notes:
1. Untranslatable words are replaced with asterisks (****).
2. Texts in the figures are not translated and shown as it is.

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CLAIMS

[Claim(s)]
[Claim 1] In the procedure of making cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase act at least, and measuring high density lipoprotein (HDL)-cholesterol in a sample The measuring method of the HDL-cholesterol which carries out making albumin add and exist and performing said enzyme reaction to another ** to albumin of sample origin with the feature.
[Claim 2] In the sample to contain, HDL Poly anion, divalent metal salt, a nonionic surfactant, In the procedure of detecting the substance which cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act; and is consumed, or the substance to generate, and measuring HDL-cholesterol in a sample The measuring method of the HDL-cholesterol according to claim 1 characterized by making albumin add and exist and carrying out said enzyme reaction to said system of reaction with albumin of sample origin at another **.
[Claim 3] The measuring method of HDL-cholesterol according to claim 2 which poly anion is sulfation polysaccharide and is one or more sorts as which a nonionic surfactant is chosen from an n-octyl beta-glucoside, an n-octyl beta-thio glucoside, and an n-heptyl beta-thio glucoside.
[Claim 4] In the reagent for making cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase act at least, and measuring high density lipoprotein (HDL)-cholesterol in a sample Furthermore, the reagent for measurement of the HDL-cholesterol characterized by making albumin live together.
[Claim 5] Poly anion, divalent metal salt, a nonionic surfactant, cholesterol esterase, In the reagent for measurement of the HDL-cholesterol which consists of a constituent for detecting cholesterol oxidase or a cholesterol dehydrogenase, the substance consumed, or the substance to generate Furthermore, the reagent for measurement of the HDL-cholesterol according to claim 4 characterized by making albumin live together.
[Claim 6] The reagent for measurement of the HDL-cholesterol according to claim 5 which is equipped with the following and characterized by making albumin live together into the first reagent. Poly anion, divalent metal salt, the first reagent containing a nonionic surfactant The second reagent which contains cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase at least
[Claim 7] Claim 5 which poly anion is sulfation polysaccharide and is one or more sorts as which a nonionic surfactant is chosen from an n-octyl beta-glucoside, an n-octyl beta-thio glucoside, and an n-heptyl beta-thio glucoside, the reagent for measurement of HDL-cholesterol given in 6.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Industrial Application] This invention relates to the reagent for measurement of the measuring method of high density lipoprotein (HDL)-cholesterol, and HDL-cholesterol.
[0002]
[Description of the Prior Art] Importance is attached to the cholesterol contained in each lipid fraction in plasma or serum as a diagnostic material in which the danger of atherosclerosis or myocardial infarction is shown in recent years. The sizes as lipid complex particles differ, the ultracentrifugal method which is a separation method using the difference of specific gravity is followed, and the lipid fractions of serum are chylomicrons and Very low density lipoprotein (Very low density lipoprotein; it is also called Following VLDL), respectively, It is classified by four kinds of low density lipoprotein (Low density lipoprotein; it is also called Following LDL) and high density lipoprotein (High density lipoprotein; it is also called Following HDL). Each lipid fraction is divided roughly into apolipoprotein and a lipid, and the lipid consists of separated type cholesterol, cholesterolester, a triglyceride, and phospholipid further. For this reason, measurement of cholesterol is performed about both separated type and ester type.
[0003] In the everyday clinical test, although measurement of the total cholesterol by enzymatic process was widely performed using the autoanalyzer, since it was required to pretreat a sample (fractionation and separation operation), about measurement of HDL-cholesterol, the spread of the automatic analysis measurement (automation) by enzymatic process was behind. As a pretreatment of this sample, various sedimentation methods are performed and For example, phosphotungstic acid and ionized magnesium, Dextran sulfate and ionized magnesium, A heparin, calcium ion, or manganese ion (M.) Burstein and H. R.Scholnick; Adv.Lipid Res., 11, 67, 1973, G.R.Warnick et al.; Clin.Chem., 25, 596, 1979, Or pressure of business of the procedure of adding a polyethylene glycol, settling LDL etc. and using a supernatant as a specimen by centrifugal operation is carried out. In detail, when the phosphotungstic acid and ionized magnesium are used as a precipitant, a sample (serum and plasma) is added to the solution containing these, and let lipid fractions other than HDL be insoluble complexes. Except for precipitation, the supernatants containing HDL are collected by carrying out centrifugal separation of this. Measurement of HDL by which fractionation was carried out by an automatic analysis system is attained with the enzyme reagent for total measurement cholesterol. Moreover, set to the immunization (C-C. Heuck, et al.Clin.Chem.31; 252, 1985). The immune body to apolipoprotein B (not contained in HDL) is added to a sample (serum and plasma) as ********, and lipid hula KUSHO other than HDL is settled. After carrying out fractionation like the following, HDL-cholesterol in a supernatant can be measured for the first time. Thus, there was a fault that the conventional procedure took each much processes and time.
[0004] The report is issued these days about the measuring method which does not need these fractionation operation (for example, a JP,6-16720,B number, a JP,7-34760,B number, JP,58-165800,A No. each gazette, international application number PCT/JP 95/00378). That is, it is enzymatic process for the total measurement cholesterol mainly used from before, Cholesterol esterase hydrolyzes a cholesterol ester and cholesterol which is this enzyme reaction product [with cholesterol oxidase] Under existence of a suitable oxidizability color fixative, make the hydrogen peroxide which is made oxidized using a dissolved oxygen and is generated color it by a peroxidase reaction, and [carry out

colorimetric measurement or] Or the procedure of measuring the dissolved oxygen amount consumed in the case of the oxidation reaction by the aforementioned cholesterol oxidase with an oxygen electrode was known.

[0005] For example, according to the description of each aforementioned patent journal, in the aforementioned system of reaction, existence of the polyethylene oxide machine content surface-active agent of a non-ion system is important for the activity manifestation of cholesterol esterase with bile salt, and it is supposed without this surface-active agent that activity will not be discovered. And in JP,H6-16720,B, it is to this bile salt, Since it is effective in making the cholesterol which a lipid melts only the chylomicron which is the lipoprotein which has comparatively slight protein in Toyotomi, and VLDL and LDL, and contains in it participate in an enzyme reaction, Take the initiative in measurement of HDL-cholesterol, this is made to react, subsequently the aforementioned surface-active agent is added, and the procedure of carrying out fractionation measurement of the HDL-cholesterol specifically is indicated by by making cholesterol and enzyme which it has in an HDL fraction react. Moreover, the thing for which the enzyme to be used is further made into cholesterol esterase of pancreas origin in the same system as the above at JP,H7-34760,B, and the anti-LDL immune body is added to the system of reaction, The complex by an antigen-antibody reaction is made to form between apolipoprotein B which is the main composition protein of LDL or VLDL, and said immune body, and the device which raises the singularity over an HDL fraction by checking a reaction with the enzyme concerned synthetically is performed.

[0006] Moreover, international application number PCT/JP95/00378 are lipoprotein other than high density lipoprotein (HDL). The cholesterol esterase by which chemical modification was carried out to the sample which contains HDL under existence of the reagent made to condense, The cholesterol oxidase or the cholesterol dehydrogenase by which chemical modification was carried out by which chemical modification was carried out is made to act, and the assay of cholesterol in HDL smoothly characterized by things is indicated in a fixed quantity of hydrogen peroxide to generate or reduction type coenzymes.

[0007] Moreover, H.Sugiuchi and others has reported the new procedure which applied international application number PCT/JP95/00378, and was adapted for the automatic analysis machine (Clin.Chem., 41, 717, 1995). That is, the enzyme which made combine it and carried out chemical modification of the polyethylene glycol to the enzyme (cholesterol esterase and cholesterol oxidase) concerned to be used, and polymer-ized it to it is used. Furthermore, the cyclodextrin inductor it is supposed in addition to the aforementioned polymer-ized enzyme that there are various lipid hula SHON and compatibility (specifically) It is indicated that a complex can be made to form to lipid fractions other than HDL by making sulfation alpha-cyclodextrin live together. Since this complex cannot undergo the reaction by said polymer-ized enzyme easily, it can measure an HDL fraction specifically.

[0008]

[Problem(s) to be Solved by the Invention] However, by the above-mentioned conventional procedure, an immune body is newly added to a reagent, or reaction time takes 20 minutes or more, In order the action to the automatic analysis machine currently used every day on manufacture cost or measurement operation is inadequate or to use the enzyme which carried out chemical modification New problems, such as an increase in the new process of a modification of enzyme, management of the degree of refining of an enzyme preparation and control of the enzyme activity change by the grade difference of chemical modification, management, and also maintenance of the stability of a modification enzyme,

will be accompanied. Moreover, imperfection cannot be denied in fractionation by use of only a surface-active agent. this invention person etc. sets into the conventional measuring method and the conventional reagent for measurement of HDL-cholesterol in the present clinical test examination in view of the point that measurement by the autoanalyzer which is a quick and simple means is mainstream. This invention was completed as a result of repeating research wholeheartedly for the purpose of development of the procedure by which it is simple operation and a highly precise measurement result is obtained, without performing centrifugal operation of a sample (serum or plasma).
[0009]
[Means for Solving the Problem] Therefore, this invention is set to the procedure of making cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase act at least, and measuring high density lipoprotein (HDL)-cholesterol in a sample. It is related with the measuring method of the HDL-cholesterol which carries out making albumin add and exist and performing said enzyme reaction to another ** to albumin of sample origin with the feature. Furthermore, this invention is set into the reagent for making cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase act at least, and measuring high density lipoprotein (HDL)-cholesterol in a sample. Furthermore, it is related also with the reagent for measurement of the HDL-cholesterol characterized by making albumin live together. This invention is explained in detail hereafter.
[0010] The feature of this invention is set for the measuring method of conventional HDL-cholesterol. If albumin is made to exist in the system of reaction artificially independently with albumin originating in living body liquid samples, such as serum and plasma It is related with the reaction of the enzyme (cholesterol esterase, the cholesterol oxidase, or cholesterol dehydrogenase) and lipid fraction content cholesterol which are used in the measuring method of HDL-cholesterol. It is based on the new knowledge of checking a reaction with cholesterol of LDL and a VLDL fraction although albumin does not influence a reaction with HDL-cholesterol.
[0011] In this invention, especially as a sample, it is the living body liquid of mammalian (especially Homo sapiens), and serum or plasma can specifically be used as it is. In this invention when contacting the aforementioned biological sample to cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase What is necessary is for albumin of sample origin to be making albumin exist artificially independently, and just to react the starting HDL-cholesterol measurement, after making a reaction with said enzyme, LDL cholesterol, and VLDL cholesterol check.
[0012] In the following description, lipoprotein and condensation reagents other than HDL assume meeting status, saying "lipoprotein other than HDL is made to condense", and the status that the reaction of this lipoprotein and enzyme is checked is said. [the system of reaction and the concrete target which are made to condense lipoprotein other than HDL and measure HDL-cholesterol] [the condensation reagent which makes LDL, VLDL, and the chylomicrons which are lipoprotein other than HDL condense, i.e., the condensation reagent which consists of poly anion and divalent metal salt, and a concrete target] dextran sulfate or its salt, and a polyethylene glycol -- passing -- Palin or its salt -- When this invention procedure is applied to measurement of HDL-cholesterol using the well-known condensation reagent which consists of the phosphotungstic acid, its salt or such combination, and divalent metal salt, Albumin is made to live together when making a sample, cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase contact. Or when making a sample, cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase contact, Albumin is made to live together beforehand, a reaction with cholesterol of LDL and a VLDL fraction is checked, it becomes

possible to suspend the reaction of LDL and a VLDL fraction completely, and only cholesterol of an HDL fraction can be alternatively measured with sufficient precision.

[0013] Since albumin is originally contained also in serum (plasma), a small amount of albumin of sample origin exists in this system of reaction. However, in order to check the reaction of lipoprotein other than HDL, cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase, it is checked in the following working examples that albumin of sample origin is inadequate. Preferably the amount of albumin in the system of reaction 0.01 to 20.0weight % 0.1 to 20.0 weight %, It is indispensable to make 0.5 to 10.0weight % of albumin exist more preferably, and in order to control within the limits of this, it is necessary with albumin of sample origin for another ** to add albumin.

[0014] [in the case of the system of reaction which is made to condense lipoprotein other than HDL and measures HDL-cholesterol] by contacting albumin, poly anion, divalent metal salt, and a nonionic surfactant and a sample for not operating centrifugal separation etc. Although LDL-cholesterol and VLDL-cholesterol, and enzyme in a specimen do not react, they come to react with HDL-cholesterol and enzyme. Subsequently, cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act. A well-known means can detect the substance (for example, coenzyme) consumed by the enzyme reaction of HDL-cholesterol and each of said enzyme, or the substance (for example, hydrogen peroxide) generated, and a fixed quantity of HDL-cholesterol can be carried out. For example, the hydrogen peroxide generated under existence of a suitable oxidizability color fixative and a peroxidase is made to color, and what is necessary is just to carry out colorimetric measurement in spectroscopy, in detecting hydrogen peroxide. Moreover, when using a cholesterol dehydrogenase, NAD(P) H generated from NAD (P) can be detected by acting as a monitor in spectroscopy on the wavelength of 340nm.

[0015] Hydrogen peroxide is a well-known procedure and, for example, can be made to color by the reaction of a peroxidase under existence of a suitable oxidizability color fixative. [measurement 3-high draw ****- 2 and 4, 6-triiodo benzoic acid (HTIBA), and N-ethyl N-sulfopropyl meta toluidine (ESPT) and 4-aminoantipyrine (4-AP) are suitable, and according to an autoanalyzer] as an oxidizability color fixative What is necessary is just to measure the absorbance near the wavelength of 510nm (when using HTIBA), or in near 546nm (when using ESPT).

[0016] Next, the reagent which made albumin live together into the conventional reagent for HDL-cholesterol measurement is explained. This invention is applicable if it is the reagent which cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act in principle, and measures HDL-cholesterol. In the reagent which is made to condense lipoprotein other than HDL for example, and specifically measures HDL-cholesterol [the condensation reagent which makes lipoprotein other than HDL condense, i.e., the condensation reagent which consists of poly anion and divalent metal salt, and a concrete target] dextran sulfate or its salt, and a polyethylene glycol -- passing -- Palin or its salt -- The condensation reagent which consists of the phosphotungstic acid, its salt or such combination, and divalent metal salt, Furthermore, a nonionic surfactant, cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase, What is necessary is just to make albumin exist in the constituent for detecting the substance (for example, coenzyme) consumed by an enzyme reaction, or the substance (for example, hydrogen peroxide) generated.

[0017] The system of reaction is made to contain in a reagent 0.1 to 20.0weight % preferably, 0.01 to 20.0weight %, as albumin, so that it may become 0.5 to 10.0 weight % more preferably. If there is less albumin concentration than 0.01 weight %, the prevention effect is inadequate, if 10.0 weight % is exceeded, the viscosity of a reagent will become high, and the problem of causing aggravation of the

reproducibility of measurement arises. Moreover, the origin in particular of albumin is not limited but can also use the thing produced in genetic engineering besides the mammalian origins, such as Homo sapiens, a cow, a sheep, and a horse. In the case of the above-mentioned reagent, as poly anion used together with albumin, sulfation polysaccharide is desirable, and in order that especially dextran sulfate may check the reaction of lipoprotein other than HDL, and enzyme effectively, it is desirable to use dextran sulfate. as dextran sulfate -- the system of reaction -- 1microM-500microM -- desirable -- dextran sulfate of the molecular weights 10000-500000 of 5.0micro M-100microM, or its salt -- Or 10micro is made to contain in a reagent M to 5 mM, so that it may become dextran sulfate of the molecular weights 1000-10000 of 50microM - 1mM, or its salt preferably. If there is less dextran sulfate concentration than a minimum, the prevention effect is inadequate, and if a maximum is exceeded, the problem of the reactant fall by prevention of enzyme will arise. As divalent metal salt, it is magnesium salt, calcium salt, and manganese salt, and can use as salt as water-soluble mineral salt (for example, chlorides, a bromide, or an iodide), for example, a halide, a sulfation thing, water-soluble organic salt, for example, acetate, or citrate. The system of reaction is made to contain in a reagent one to 100 mM, as a divalent metal, so that it may become the concentration of 5 - 50mM preferably. If there is less concentration of divalent metal salt than a minimum, the prevention effect is inadequate, and if a maximum is exceeded, the problem of the reactant fall by prevention of salting out of the metal by preservation or enzyme will arise.

[0018] Although a nonionic surfactant at large [well-known] can be used as a nonionic surfactant In the case of the above-mentioned reagent, especially as a nonionic surfactant used together with albumin An n-octyl beta-glucoside, In order that an n-octyl beta-thio glucoside and an n-heptyl beta-thio glucoside may check the reaction of lipoprotein other than HDL, and enzyme suitably, it is desirable to use one or more sorts of things chosen from these. When the concentration in the system of reaction is an n-octyl beta-glucoside, it is made to contain in a reagent 0.01 to 2.0%, so that it may become 0.1 to 1.0% preferably. In the case of an n-octyl beta-thio glucoside, it uses at 0.05 to 0.5% preferably 0.01 to 1.0%. In the case of an n-heptyl beta-thio glucoside, it uses at 0.1 to 1.0% preferably 0.01 to 2.0%. Or you may mix and use these by said concentration within the limits. If there is less concentration of said nonionic surfactant than a minimum, reacting with enzyme will become inadequate, and if a maximum is exceeded, the problem that the prevention effect over HDL-cholesterol falls will arise.

[0019] Either the enzyme which was made to combine a polyethylene glycol (PEG) etc. and carried out chemical modification as enzyme, such as cholesterol esterase and cholesterol oxidase, or the enzyme which has not carried out chemical modification can be used. The origin of enzyme is not limited, either but the microbe of Pseudomonas and cholesterol esterase of the pancreas origin of a cow or a pig can be used as cholesterol esterase, for example. Moreover, as cholesterol oxidase, the cholesterol oxidase of the microbe origin of Streptomyces or the Nocardia group can be used, for example. The same may be said of a cholesterol dehydrogenase, for example, the thing of microbe origin can be used. although the amount of addition in particular of those enzyme is not limited, either -- 0.1u/ml-20u/ml -- it is 0.2u/ml-10u/ml more preferably.

[0020] Cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act. What is necessary is just to use a well-known constituent, in order to detect the substance (for example, coenzyme) consumed by the enzyme reaction of HDL-cholesterol and each of said enzyme, or the substance (for example, hydrogen peroxide) generated. For example, when detecting hydrogen peroxide, the hydrogen peroxide generated under existence of a suitable oxidizability color fixative and a

peroxidase can be made to be able to color, and colorimetric measurement can be made in spectroscopy, for example, it can be made to color by the reaction of a peroxidase under existence of a suitable oxidizability color fixative. As an oxidizability color fixative, 3-high draw ****- 2 and 4, 6-triiodo benzoic acid (HTIBA), and N-ethyl N-sulfopropyl meta toluidine (ESPT) and 4-aminoantipyrine (4-AP) are suitable. HTIBA and ESPT are the concentration ranges of 0.1mM - 5mM, and 4-AP can be made to contain suitably in the concentration range of 0.05mM - 2mM.

[0021] The reagent for HDL-cholesterol measurement of this invention can be made into 2 reagent system according to the autoanalyzer used widely now. In this case, the first reagent is made to contain albumin, poly anion (for example, dextran sulfate), divalent metal salt, and a nonionic surfactant, and the second reagent is made to contain cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase. As a buffer of the first reagent and the second reagent, good buffer solution, such as a phosphate buffer solution, BES, HEPES, and PIPES, Tris buffer, an imidazole buffer, etc. can be used. As concentration of buffer solution, it is 20 - 500mM preferably ten to 1000 mM. Moreover, pH of those buffer solution can be suitably chosen within 5.0-9.0, and pH 6.0 to 8.0 desirable limits with good prevention with cholesterol of LDL and cholesterol of VLDL, and enzyme.

[0022] It will be as follows if the system of reaction in the case of measuring HDL-cholesterol is typically shown using this invention reagent of a 2 reagent system.

The first reagent (albumin, poly anion, divalent metal salt, nonionic surfactant)

+ Specimen (serum/plasma)

The reaction prevention-ized second reagent of **LDL, VLDL, and enzyme (enzyme and coloring system composition ingredient content)

(Cholesterol esterase reaction)

Cholesterol ester +H2 O-> cholesterol + fatty acid (1)

(Cholesterol oxidase reaction)

Cholesterol +O2 ->delta 4-cholesten-3-one +H2O2 (2)

(Peroxidase reaction)

H2O2+ oxidizability color fixative -> oxidization condensation product (3)

** spectrometry [0023] Although the hydrogen peroxide mainly generated by said reaction formula (2) is measured with colorimetric method in measurement by an autoanalyzer, it can use similarly not only by a reaction in these solution but by the dry type system of measurement (dry chemistry) according to the piece of a filter paper test etc. for example. Moreover, hydrogen peroxide can also measure the oxidation potential difference to generate electrochemically by making it react under existence of suitable mediators, such as a potassium ferrocyanide, and a peroxidase. On the other hand, the substance consumed by an enzyme reaction, for example, the oxygen consumed by said reaction formula (2), (dissolved oxygen) can also be conventionally measured by a well-known procedure, for example, an oxygen electrode. Moreover, as a compound generated by the enzyme reaction, you may measure fatty acid which is the product of said reaction formula (1), or delta 4-cholesten-3-one which is the product of said reaction formula (2) by a suitable procedure, for example besides the aforementioned hydrogen peroxide.

[0024]

[Function] In [although not limited to the following explanation] this invention, The reaction of the enzyme (cholesterol esterase and the cholesterol oxidase, or cholesterol dehydrogenase) and lipid fraction content cholesterol which are used in the measuring method of HDL-cholesterol is faced. The

compound used as said conventional condensation reagent shows compatibility directly to the apolipoprotein of a lipid fraction, or is indirectly considered to carry out an interaction to enzyme by the reaction time of cholesterol of a lipid fraction, and enzyme. Namely, although each lipid fraction serves as a lipid complex which consists of a lipid and apolipoprotein The physicochemical quality and the quantitative (quantity contained in a specimen) difference arising from the difference of the difference in the lipid percentage, and the types (A-1, A-1, B-100, B-48, C, E, etc.) of apolipoprotein It is identified. Since the difference in the type (A-1, A-2, and the latter are B-100, C, and E in the former) of apolipoprotein different most greatly between an HDL fraction, LDL, and a VLDL fraction is clear, the method of using the immune body to apolipoprotein has also been developed conventionally. It is the feature for this conventional method to mix with the immune body to the apolipoprotein B and C at a sample, and to make an immune complex form. Since only HDL-cholesterol will react with enzyme if enzyme is added next in order to cause enzyme reaction prevention, this immune complex can measure only HDL-cholesterol. However, difficultly, since muddiness of the immune complex itself was remarkable, maintaining uniformly the reactivity of the immune body which can form an immune complex had the fault that an error became large on the occasion of the colorimetric measurement for cholesterol measurement.

[0025] On the other hand, at this invention, it is addition of albumin. Said conventional condensation reagent acts on mutual [centering on apolipoprotein / a lipid complex and mutual]. Do not need the chemical modification of enzyme etc. but are based on cholesterol esterase and cholesterol oxidase. Can change the reactivity over cholesterol of each lipid fraction specifically, and, moreover, [this invention procedure] Unlike an immune body, it is a stable compound and it is not necessary to take into consideration further about new problems, such as an increase in the new process accompanying a modification of enzyme, management of the degree of refining of an enzyme preparation and control of the enzyme activity change by the grade difference of chemical modification, management, and also maintenance of the stability of a modification enzyme.

[0024]

[Example] Hereafter, although a working example explains this invention concretely, these do not limit the range of this invention.

Working example 1: Fractionation of the lipid fraction by the fractionation ultracentrifugal method of the lipid fraction by an ultracentrifugal method was performed according to Akio Kudo's (arteriosclerosis, 6, 39, 1978) etc. procedure. 16mg of EDTA sodium salt, 4g of sucrose, 3.2g of potassium bromide, and 0.8g of sodium chloride were added to 16ml of pooled serum, and, specifically, it dissolved in it. Apart from this, three kinds of specific gravity liquid was created. That is, the specific gravity liquid of specific gravity 1.21 dissolved 20g of sucrose, 15g of potassium bromide, and 5g of sodium chloride in 100ml of purified water, and was prepared. It mixed with the specific gravity liquid of specific gravity 1.063, and it prepared said 30ml of specific gravity liquid and 70ml of purified water of specific gravity 1.21. Moreover, the specific gravity liquid of specific gravity 1.006 dissolved in 97.5ml of purified water, and prepared 2.5g of sucrose. 1.9ml of the above-mentioned serum was put into the centrifugation vessel of 10ml capacity, 0.8ml of specific gravity liquid of specific gravity 1.21 was calmly stratified with the syringe in this upper layer, and centrifugality of the centrifugation vessel was carried out at 10 degrees C for 50000rpm 20 hours. After the end of centrifugal processing, although all the with a specific gravity of 1.21 or less lipid fractions gathered for the top layer part, 1.6ml of specific gravity liquid of specific gravity 1.063 and 2ml of specific gravity liquid of specific gravity

1.006 were further stratified on this top layer part. Centrifugality of this centrifugation vessel was further carried out at 50000rpm for 4 hours, and each lipid fractions were collected. Cold storage of each fraction was carried out to the physiological salt solution after overnight dialysis (under refrigeration).
[0025] Working example 2: 0.6ml of solution containing each poly anion shown in the example table 1 of search of a reaction repressor, 0.15ml of bis-tris buffer solution of 250mM containing ESPT of the magnesium chloride of 100mM, 1.0% of n-OTG, and 5mM (pH 7.0), 20microl of HDL, 10microl of LDL, and 10microl of VLDL were respectively mixed among the lipid fractions obtained in the working example 1 as a sample, and it warmed for 5 minutes at 37 degrees C. They are 4-AP of 0.5mM, 20micro ag [/ml] POD, and CHE (Pseudomonas origin) and CHO (Nocardia origin) of each 5u/ml to this. After carrying out mixed churning and making 0.25ml of bis-tris buffer solution (pH 7.0) of included 50mM react for 5 minutes at 37 degrees C, the absorbance in the wavelength of 546nm was measured. Moreover, same operation was performed in each poly anion content solution using what added albumin by the concentration shown in Table 2. Independently, using the purified water which does not contain poly anion and albumin, the same operation as the above was performed and absorbance was measured. Absorbance in case poly anion and albumin are not included was set to 100, and it asked for the decreasing rate of the absorbance when using what added albumin to each poly anion and each poly anion as a reaction prevention rate to each lipid fraction. A result is shown in Table 1 and Table 2. In addition, the cable address of each substance is a following meaning.
DS:dextran sulfate ALB: -- a serum albumin.
P: Phosphotungstic-acid sodium HP: It passes and is PARIN sodium PEG:polyethylene glycol n-OTG:n-octyl beta-D-thio glucoside CHE:cholesterol esterase CHO:cholesterol oxidase POD:peroxidase ESPT: N-ethyl N-sulfopropyl meta toluidine. [0026]
[Table 1]
Poly anion concentration HDL prevention rate LDL prevention rate VLDL prevention rate DS0.5mM2% 64%31%P0.5mM1%28%18%H.P. 50U/ml 1% 35% 24%PEG 0.5mM 0% 5% 3% [0027]
[Table 2]

TECHNICAL FIELD

[Industrial Application] This invention relates to the reagent for measurement of the measuring method of high density lipoprotein (HDL)-cholesterol, and HDL-cholesterol.

PRIOR ART

[Description of the Prior Art] Importance is attached to the cholesterol contained in each lipid fraction in plasma or serum as a diagnostic material in which the danger of atherosclerosis or myocardial infarction is shown in recent years. The sizes as lipid complex particles differ, the ultracentrifugal method which is a separation method using the difference of specific gravity is followed, and the lipid fractions of serum are chylomicrons and Very low density lipoprotein (Very low density lipoprotein; it is also called Following VLDL), respectively, It is classified by four kinds of low density lipoprotein (Low density lipoprotein; it is also called Following LDL) and high density lipoprotein (High density lipoprotein; it is also called Following HDL). Each lipid fraction is divided roughly into apolipoprotein and a lipid, and the lipid consists of separated type cholesterol, cholesterolester, a triglyceride, and phospholipid further. For this reason, measurement of cholesterol is performed about both separated type and ester type.

[0003] In the everyday clinical test, although measurement of the total cholesterol by enzymatic process was widely performed using the autoanalyzer, since it was required to pretreat a sample (fractionation and separation operation), about measurement of HDL-cholesterol, the spread of the automatic analysis measurement (automation) by enzymatic process was behind. As a pretreatment of this sample, various sedimentation methods are performed and For example, phosphotungstic acid and ionized magnesium, Dextran sulfate and ionized magnesium, A heparin, calcium ion, or manganese ion (M.) Burstein and H. R.Scholnick; Adv.Lipid Res., 11, 67, 1973, G.R.Warnick et al.; Clin.Chem., 25, 596, 1979, Or pressure of business of the procedure of adding a polyethylene glycol, settling LDL etc. and using a supernatant as a specimen by centrifugal operation is carried out. In detail, when the phosphotungstic acid and ionized magnesium are used as a precipitant, a sample (serum and plasma) is added to the solution containing these, and let lipid fractions other than HDL be insoluble complexes. Except for precipitation, the supernatants containing HDL are collected by carrying out centrifugal separation of this. Measurement of HDL by which fractionation was carried out by an automatic analysis system is attained with the enzyme reagent for total measurement cholesterol. Moreover, set to the immunization (C-C. Heuck, et al.Clin.Chem.31, 252, 1985). The immune body to apolipoprotein B (not contained in HDL) is added to a sample (serum and plasma) as ********, and lipid hula KUSHO other than HDL is settled. After carrying out fractionation like the following, HDL-cholesterol in a supernatant can be measured for the first time. Thus, there was a fault that the conventional procedure took each much processes and time.

[0004] The report is issued these days about the measuring method which does not need these fractionation operation (for example, a JP,6-16720,B number, a JP,7-34760,B number, JP,58-165800,A No. each gazette, international application number PCT/JP 95/00378). That is, it is enzymatic process for the total measurement cholesterol mainly used from before, Cholesterol esterase hydrolyzes a cholesterol ester and cholesterol which is this enzyme reaction product [with cholesterol oxidase] Under existence of a suitable oxidizability color fixative, make the hydrogen peroxide which is made oxidized using a dissolved oxygen and is generated color it by a peroxidase reaction, and [carry out colorimetric measurement or] Or the procedure of measuring the dissolved oxygen amount consumed in the case of the oxidation reaction by the aforementioned cholesterol oxidase with an oxygen electrode was known.

[0005] For example, according to the description of each aforementioned patent journal, in the aforementioned system of reaction, existence of the polyethylene oxide machine content surface-active

agent of a non-ion system is important for the activity manifestation of cholesterol esterase with bile salt, and it is supposed without this surface-active agent that activity will not be discovered. And in JP,H6-16720,B, it is to this bile salt, Since it is effective in making the cholesterol which a lipid melts only the chylomicron which is the lipoprotein which has comparatively slight protein in Toyotomi, and VLDL and LDL, and contains in it participate in an enzyme reaction, Take the initiative in measurement of HDL-cholesterol, this is made to react, subsequently the aforementioned surface-active agent is added, and the procedure of carrying out fractionation measurement of the HDL-cholesterol specifically is indicated by by making cholesterol and enzyme which it has in an HDL fraction react. Moreover, the thing for which the enzyme to be used is further made into cholesterol esterase of pancreas origin in the same system as the above at JP,H7-34760,B, and the anti-LDL immune body is added to the system of reaction, The complex by an antigen-antibody reaction is made to form between apolipoprotein B which is the main composition protein of LDL or VLDL, and said immune body, and the device which raises the singularity over an HDL fraction by checking a reaction with the enzyme concerned synthetically is performed.

[0006] Moreover, international application number PCT/JP95/00378 are lipoprotein other than high density lipoprotein (HDL). The cholesterol esterase by which chemical modification was carried out to the sample which contains HDL under existence of the reagent made to condense, The cholesterol oxidase or the cholesterol dehydrogenase by which chemical modification was carried out by which chemical modification was carried out is made to act, and the assay of cholesterol in HDL smoothly characterized by things is indicated in a fixed quantity of hydrogen peroxide to generate or reduction type coenzymes.

[0007] Moreover, H.Sugiuchi and others has reported the new procedure which applied international application number PCT/JP95/00378, and was adapted for the automatic analysis machine (Clin.Chem., 41, 717, 1995). That is, the enzyme which made combine it and carried out chemical modification of the polyethylene glycol to the enzyme (cholesterol esterase and cholesterol oxidase) concerned to be used, and polymer-ized it to it is used. Furthermore, the cyclodextrin inductor it is supposed in addition to the aforementioned polymer-ized enzyme that there are various lipid hula SHON and compatibility (specifically) It is indicated that a complex can be made to form to lipid fractions other than HDL by making sulfation alpha-cyclodextrin live together. Since this complex cannot undergo the reaction by said polymer-ized enzyme easily, it can measure an HDL fraction specifically.

EFFECT OF THE INVENTION

[Effect of the Invention] Highly precise measurement of HDL-cholesterol can be performed by simple operation, without performing centrifugal operation of a sample (serum or plasma).

TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] However, by the above-mentioned conventional procedure, an immune body is newly added to a reagent, or reaction time takes 20 minutes or more, In order the action to the automatic analysis machine currently used every day on manufacture cost or measurement operation is inadequate or to use the enzyme which carried out chemical modification New problems, such as an increase in the new process of a modification of enzyme, management of the degree of refining of an enzyme preparation and control of the enzyme activity change by the grade difference of chemical modification, management, and also maintenance of the stability of a modification enzyme, will be accompanied. Moreover, imperfection cannot be denied in fractionation by use of only a surface-active agent. this invention person etc. sets into the conventional measuring method and the conventional reagent for measurement of HDL-cholesterol in the present clinical test examination in view of the point that measurement by the autoanalyzer which is a quick and simple means is mainstream. This invention was completed as a result of repeating research wholeheartedly for the purpose of development of the procedure by which it is simple operation and a highly precise measurement result is obtained, without performing centrifugal operation of a sample (serum or plasma).

MEANS

[Means for Solving the Problem] Therefore, this invention is set to the procedure of making cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase act at least, and measuring high density lipoprotein (HDL)-cholesterol in a sample. It is related with the measuring method of the HDL-cholesterol which carries out making albumin add and exist and performing said enzyme reaction to another ** to albumin of sample origin with the feature. Furthermore, this invention is set into the reagent for making cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase act at least, and measuring high density lipoprotein (HDL)-cholesterol in a sample. Furthermore, it is related also with the reagent for measurement of the HDL-cholesterol characterized by making albumin live together. This invention is explained in detail hereafter.

[0010] The feature of this invention is set for the measuring method of conventional HDL-cholesterol. If albumin is made to exist in the system of reaction artificially independently with albumin originating in living body liquid samples, such as serum and plasma It is related with the reaction of the enzyme (cholesterol esterase, the cholesterol oxidase, or cholesterol dehydrogenase) and lipid fraction content cholesterol which are used in the measuring method of HDL-cholesterol. It is based on the new knowledge of checking a reaction with cholesterol of LDL and a VLDL fraction although albumin does not influence a reaction with HDL-cholesterol.

[0011] In this invention, especially as a sample, it is the living body liquid of mammalian (especially Homo sapiens), and serum or plasma can specifically be used as it is. In this invention when contacting the aforementioned biological sample to cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase What is necessary is for albumin of sample origin to be making albumin exist artificially independently, and just to react the starting HDL-cholesterol measurement, after making a reaction with said enzyme, LDL cholesterol, and VLDL cholesterol check.

[0012] In the following description, lipoprotein and condensation reagents other than HDL assume meeting status, saying "lipoprotein other than HDL is made to condense", and the status that the reaction of this lipoprotein and enzyme is checked is said. [the system of reaction and the concrete target which are made to condense lipoprotein other than HDL and measure HDL-cholesterol] [the condensation reagent which makes LDL, VLDL, and the chylomicrons which are lipoprotein other than HDL condense, i.e., the condensation reagent which consists of poly anion and divalent metal salt, and a concrete target] dextran sulfate or its salt, and a polyethylene glycol -- passing -- Palin or its salt -- When this invention procedure is applied to measurement of HDL-cholesterol using the well-known condensation reagent which consists of the phosphotungstic acid, its salt or such combination, and divalent metal salt, Albumin is made to live together when making a sample, cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase contact. Or when making a sample, cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase contact, Albumin is made to live together beforehand, a reaction with cholesterol of LDL and a VLDL fraction is checked, it becomes possible to suspend the reaction of LDL and a VLDL fraction completely, and only cholesterol of an HDL fraction can be alternatively measured with sufficient precision.

[0013] Since albumin is originally contained also in serum (plasma), a small amount of albumin of sample origin exists in this system of reaction. However, in order to check the reaction of lipoprotein other than HDL, cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase, it is checked in the following working examples that albumin of sample origin is inadequate. Preferably the

amount of albumin in the system of reaction 0.01 to 20.0weight % 0.1 to 20.0 weight %, It is indispensable to make 0.5 to 10.0weight % of albumin exist more preferably, and in order to control within the limits of this, it is necessary with albumin of sample origin for another ** to add albumin.
[0014] [in the case of the system of reaction which is made to condense lipoprotein other than HDL and measures HDL-cholesterol] by contacting albumin, poly anion, divalent metal salt, and a nonionic surfactant and a sample for not operating centrifugal separation etc. Although LDL-cholesterol and VLDL-cholesterol, and enzyme in a specimen do not react, they come to react with HDL-cholesterol and enzyme. Subsequently, cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act. A well-known means can detect the substance (for example, coenzyme) consumed by the enzyme reaction of HDL-cholesterol and each of said enzyme, or the substance (for example, hydrogen peroxide) generated, and a fixed quantity of HDL-cholesterol can be carried out. For example, the hydrogen peroxide generated under existence of a suitable oxidizability color fixative and a peroxidase is made to color, and what is necessary is just to carry out colorimetric measurement in spectroscopy, in detecting hydrogen peroxide. Moreover, when using a cholesterol dehydrogenase, NAD(P) H generated from NAD (P) can be detected by acting as a monitor in spectroscopy on the wavelength of 340nm.
[0015] Hydrogen peroxide is a well-known procedure and, for example, can be made to color by the reaction of a peroxidase under existence of a suitable oxidizability color fixative. [measurement 3-high draw ****- 2 and 4, 6-triiodo benzoic acid (HTIBA), and N-ethyl N-sulfopropyl meta toluidine (ESPT) and 4-aminoantipyrine (4-AP) are suitable, and according to an autoanalyzer] as an oxidizability color fixative What is necessary is just to measure the absorbance near the wavelength of 510nm (when using HTIBA), or in near 546nm (when using ESPT).
[0016] Next, the reagent which made albumin live together into the conventional reagent for HDL-cholesterol measurement is explained. This invention is applicable if it is the reagent which cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act in principle, and measures HDL-cholesterol. In the reagent which is made to condense lipoprotein other than HDL for example, and specifically measures HDL-cholesterol [the condensation reagent which makes lipoprotein other than HDL condense, i.e., the condensation reagent which consists of poly anion and divalent metal salt, and a concrete target] dextran sulfate or its salt, and a polyethylene glycol -- passing -- Palin or its salt -- The condensation reagent which consists of the phosphotungstic acid, its salt or such combination, and divalent metal salt, Furthermore, a nonionic surfactant, cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase, What is necessary is just to make albumin exist in the constituent for detecting the substance (for example, coenzyme) consumed by an enzyme reaction, or the substance (for example, hydrogen peroxide) generated.
[0017] The system of reaction is made to contain in a reagent 0.1 to 20.0weight % preferably, 0.01 to 20.0weight %, as albumin, so that it may become 0.5 to 10.0 weight % more preferably. If there is less albumin concentration than 0.01 weight %, the prevention effect is inadequate, if 10.0 weight % is exceeded, the viscosity of a reagent will become high, and the problem of causing aggravation of the reproducibility of measurement arises. Moreover, the origin in particular of albumin is not limited but can also use the thing produced in genetic engineering besides the mammalian origins, such as Homo sapiens, a cow, a sheep, and a horse. In the case of the above-mentioned reagent, as poly anion used together with albumin, sulfation polysaccharide is desirable, and in order that especially dextran sulfate may check the reaction of lipoprotein other than HDL, and enzyme effectively, it is desirable to use dextran sulfate. as dextran sulfate -- the system of reaction -- 1microM-500microM -- desirable --

dextran sulfate of the molecular weights 10000-500000 of 5.0micro M-100microM, or its salt -- Or 10micro is made to contain in a reagent M to 5 mM, so that it may become dextran sulfate of the molecular weights 1000-10000 of 50microM - 1mM, or its salt preferably. If there is less dextran sulfate concentration than a minimum, the prevention effect is inadequate, and if a maximum is exceeded, the problem of the reactant fall by prevention of enzyme will arise. As divalent metal salt, it is magnesium salt, calcium salt, and manganese salt, and can use as salt as water-soluble mineral salt (for example, chlorides, a bromide, or an iodide), for example, a halide, a sulfation thing, water-soluble organic salt, for example; acetate, or citrate. The system of reaction is made to contain in a reagent one to 100 mM, as a divalent metal, so that it may become the concentration of 5 - 50mM preferably. If there is less concentration of divalent metal salt than a minimum, the prevention effect is inadequate, and if a maximum is exceeded, the problem of the reactant fall by prevention of salting out of the metal by preservation or enzyme will arise.

[0018] Although a nonionic surfactant at large [well-known] can be used as a nonionic surfactant In the case of the above-mentioned reagent, especially as a nonionic surfactant used together with albumin An n-octyl beta-glucoside, In order that an n-octyl beta-thio glucoside and an n-heptyl beta-thio glucoside may check the reaction of lipoprotein other than HDL, and enzyme suitably, it is desirable to use one or more sorts of things chosen from these. When the concentration in the system of reaction is an n-octyl beta-glucoside, it is made to contain in a reagent 0.01 to 2.0%, so that it may become 0.1 to 1.0% preferably. In the case of an n-octyl beta-thio glucoside, it uses at 0.05 to 0.5% preferably 0.01 to 1.0%. In the case of an n-heptyl beta-thio glucoside, it uses at 0.1 to 1.0% preferably 0.01 to 2.0%. Or you may mix and use these by said concentration within the limits. If there is less concentration of said nonionic surfactant than a minimum, reacting with enzyme will become inadequate, and if a maximum is exceeded, the problem that the prevention effect over HDL-cholesterol falls will arise.

[0019] Either the enzyme which was made to combine a polyethylene glycol (PEG) etc. and carried out chemical modification as enzyme, such as cholesterol esterase and cholesterol oxidase, or the enzyme which has not carried out chemical modification can be used. The origin of enzyme is not limited, either but the microbe of Pseudomonas and cholesterol esterase of the pancreas origin of a cow or a pig can be used as cholesterol esterase, for example. Moreover, as cholesterol oxidase, the cholesterol oxidase of the microbe origin of Streptomyces or the Nocardia group can be used, for example. The same may be said of a cholesterol dehydrogenase, for example, the thing of microbe origin can be used. although the amount of addition in particular of those enzyme is not limited, either -- 0.1u/ml-20u/ml -- it is 0.2u/ml-10u/ml more preferably.

[0020] Cholesterol esterase, cholesterol oxidase, or a cholesterol dehydrogenase is made to act. What is necessary is just to use a well-known constituent, in order to detect the substance (for example, coenzyme) consumed by the enzyme reaction of HDL-cholesterol and each of said enzyme, or the substance (for example, hydrogen peroxide) generated. For example, when detecting hydrogen peroxide, the hydrogen peroxide generated under existence of a suitable oxidizability color fixative and a peroxidase can be made to be able to color, and colorimetric measurement can be made in spectroscopy, for example, it can be made to color by the reaction of a peroxidase under existence of a suitable oxidizability color fixative. As an oxidizability color fixative, 3-high draw ****- 2 and 4, 6-triiodo benzoic acid (HTIBA), and N-ethyl N-sulfopropyl meta toluidine (ESPT) and 4-aminoantipyrine (4-AP) are suitable. HTIBA and ESPT are the concentration ranges of 0.1mM - 5mM, and 4-AP can be made to contain suitably in the concentration range of 0.05mM - 2mM.

[0021] The reagent for HDL-cholesterol measurement of this invention can be made into 2 reagent system according to the autoanalyzer used widely now. In this case, the first reagent is made to contain albumin, poly anion (for example, dextran sulfate), divalent metal salt, and a nonionic surfactant, and the second reagent is made to contain cholesterol esterase and cholesterol oxidase, or a cholesterol dehydrogenase. As a buffer of the first reagent and the second reagent, good buffer solution, such as a phosphate buffer solution, BES, HEPES, and PIPES, Tris buffer, an imidazole buffer, etc. can be used. As concentration of buffer solution, it is 20 - 500mM preferably ten to 1000 mM. Moreover, pH of those buffer solution can be suitably chosen within 5.0-9.0, and pH 6.0 to 8.0 desirable limits with good prevention with cholesterol of LDL and cholesterol of VLDL, and enzyme.
[0022] It will be as follows if the system of reaction in the case of measuring HDL-cholesterol is typically shown using this invention reagent of a 2 reagent system.
The first reagent (albumin, poly anion, divalent metal salt, nonionic surfactant)
+ Specimen (serum/plasma)
The reaction prevention-ized second reagent of **LDL, VLDL, and enzyme (enzyme and coloring system composition ingredient content)
(Cholesterol esterase reaction)
Cholesterol ester +H2 O-> cholesterol + fatty acid (1)
(Cholesterol oxidase reaction)
Cholesterol +O2 ->delta 4-cholesten-3-one +H2O2 (2)
(Peroxidase reaction)
H2O2+ oxidizability color fixative -> oxidization condensation product (3)
** spectrometry [0023] Although the hydrogen peroxide mainly generated by said reaction formula (2) is measured with colorimetric method in measurement by an autoanalyzer, it can use similarly not only by a reaction in these solution but by the dry type system of measurement (dry chemistry) according to the piece of a filter paper test etc. for example. Moreover, hydrogen peroxide can also measure the oxidation potential difference to generate electrochemically by making it react under existence of suitable mediators, such as a potassium ferrocyanide, and a peroxidase. On the other hand, the substance consumed by an enzyme reaction, for example, the oxygen consumed by said reaction formula (2), (dissolved oxygen) can also be conventionally measured by a well-known procedure, for example, an oxygen electrode. Moreover, as a compound generated by the enzyme reaction, you may measure fatty acid which is the product of said reaction formula (1), or delta 4-cholesten-3-one which is the product of said reaction formula (2) by a suitable procedure, for example besides the aforementioned hydrogen peroxide.

OPERATION

[Function] In [although not limited to the following explanation] this invention, The reaction of the enzyme (cholesterol esterase and the cholesterol oxidase, or cholesterol dehydrogenase) and lipid fraction content cholesterol which are used in the measuring method of HDL-cholesterol is faced. The compound used as said conventional condensation reagent shows compatibility directly to the apolipoprotein of a lipid fraction, or is indirectly considered to carry out an interaction to enzyme by the reaction time of cholesterol of a lipid fraction, and enzyme. Namely, although each lipid fraction serves as a lipid complex which consists of a lipid and apolipoprotein The physicochemical quality and the quantitative (quantity contained in a specimen) difference arising from the difference of the difference in the lipid percentage, and the types (A-1, A-1, B-100, B-48, C, E, etc.) of apolipoprotein It is identified. Since the difference in the type (A-1, A-2, and the latter are B-100, C, and E in the former) of apolipoprotein different most greatly between an HDL fraction, LDL, and a VLDL fraction is clear, the method of using the immune body to apolipoprotein has also been developed conventionally. It is the feature for this conventional method to mix with the immune body to the apolipoprotein B and C at a sample, and to make an immune complex form. Since only HDL-cholesterol will react with enzyme if enzyme is added next in order to cause enzyme reaction prevention, this immune complex can measure only HDL-cholesterol. However, difficultly, since muddiness of the immune complex itself was remarkable, maintaining uniformly the reactivity of the immune body which can form an immune complex had the fault that an error became large on the occasion of the colorimetric measurement for cholesterol measurement.

[0025] On the other hand, at this invention, it is addition of albumin. Said conventional condensation reagent acts on mutual [centering on apolipoprotein / a lipid complex and mutual]. Do not need the chemical modification of enzyme etc. but are based on cholesterol esterase and cholesterol oxidase. Can change the reactivity over cholesterol of each lipid fraction specifically, and, moreover, [this invention procedure] Unlike an immune body, it is a stable compound and it is not necessary to take into consideration further about new problems, such as an increase in the new process accompanying a modification of enzyme, management of the degree of refining of an enzyme preparation and control of the enzyme activity change by the grade difference of chemical modification, management, and also maintenance of the stability of a modification enzyme.

EXAMPLE

[Example] Hereafter, although a working example explains this invention concretely, these do not limit the range of this invention.

Working example 1: Fractionation of the lipid fraction by the fractionation ultracentrifugal method of the lipid fraction by an ultracentrifugal method was performed according to Akio Kudo's (arteriosclerosis, 6, 39, 1978) etc. procedure. 16mg of EDTA sodium salt, 4g of sucrose, 3.2g of potassium bromide, and 0.8g of sodium chloride were added to 16ml of pooled serum, and, specifically, it dissolved in it. Apart from this, three kinds of specific gravity liquid was created. That is, the specific gravity liquid of specific gravity 1.21 dissolved 20g of sucrose, 15g of potassium bromide, and 5g of sodium chloride in 100ml of purified water, and was prepared. It mixed with the specific gravity liquid of specific gravity 1.063, and it prepared said 30ml of specific gravity liquid and 70ml of purified water of specific gravity 1.21. Moreover, the specific gravity liquid of specific gravity 1.006 dissolved in 97.5ml of purified water, and prepared 2.5g of sucrose. 1.9ml of the above-mentioned serum was put into the centrifugation vessel of 10ml capacity, 0.8ml of specific gravity liquid of specific gravity 1.21 was calmly stratified with the syringe in this upper layer, and centrifugality of the centrifugation vessel was carried out at 10 degrees C for 50000rpm 20 hours. After the end of centrifugal processing, although all the with a specific gravity of 1.21 or less lipid fractions gathered for the top layer part, 1.6ml of specific gravity liquid of specific gravity 1.063 and 2ml of specific gravity liquid of specific gravity 1.006 were further stratified on this top layer part. Centrifugality of this centrifugation vessel was further carried out at 50000rpm for 4 hours, and each lipid fractions were collected. Cold storage of each fraction was carried out to the physiological salt solution after overnight dialysis (under refrigeration).

[0025] Working example 2: 0.6ml of solution containing each poly anion shown in the example table 1 of search of a reaction repressor, 0.15ml of bis-tris buffer solution of 250mM containing ESPT of the magnesium chloride of 100mM, 1.0% of n-OTG, and 5mM (pH 7.0), 20microl of HDL, 10microl of LDL, and 10microl of VLDL were respectively mixed among the lipid fractions obtained in the working example 1 as a sample, and it warmed for 5 minutes at 37 degrees C. They are 4-AP of 0.5mM, 20micro ag [/ml] POD, and CHE (Pseudomonas origin) and CHO (Nocardia origin) of each 5u/ml to this. After carrying out mixed churning and making 0.25ml of bis-tris buffer solution (pH 7.0) of included 50mM react for 5 minutes at 37 degrees C, the absorbance in the wavelength of 546nm was measured. Moreover, same operation was performed in each poly anion content solution using what added albumin by the concentration shown in Table 2. Independently, using the purified water which does not contain poly anion and albumin, the same operation as the above was performed and absorbance was measured. Absorbance in case poly anion and albumin are not included was set to 100, and it asked for the decreasing rate of the absorbance when using what added albumin to each poly anion and each poly anion as a reaction prevention rate to each lipid fraction. A result is shown in Table 1 and Table 2. In addition, the cable address of each substance is a following meaning.

DS:dextran sulfate ALB: -- a serum albumin.

P: Phosphotungstic-acid sodium HP: It passes and is PARIN sodium PEG:polyethylene glycol n-OTG:n-octyl beta-D-thio glucoside CHE:cholesterol esterase CHO:cholesterol oxidase POD:peroxidase ESPT: N-ethyl N-sulfopropyl meta toluidine. [0026]

[Table 1]

Poly anion concentration HDL prevention rate LDL prevention rate VLDL prevention rate DS0.5mM2%

64%31%P0.5mM1%28%18%H.P. 50U/ml 1% 35% 24%PEG 0.5mM 0% 5% 3% [0027]
[Table 2]
Poly anion Concentration Albumin (%) HDL prevention rate LDL prevention rate VLDL prevention rate DS0.5mM2%4%97%98%P0.5mM2%2%44%64%H.P.50U/ml2%3%56%34%PEG0.5mM2%1%9%7% DS0.5mM0.02%2%67%45%DS0.5mM 0.1% 2% 78% 57%DS 0.5mM 0.2% 2% 81% 68%DS0.5mM1% 2%92%90%DS0.5mM 10% 5% 94% 95%HP 0.5mM 5% 4% 65% 57% [0028] The result of Table 1 and Table 2 shows having checked LDL and VLDL enough by addition of ALB more than a fixed quantity in the system of reaction which is made to condense lipoprotein other than HDL and measures HDL-cholesterol. Especially the combination of ALB and DS was effective. Thereby, cholesterol of an HDL fraction can be correctly measured with sufficient precision.
[0029] Working example 3: As a sample, 10micro of serum l was added to 0.75ml of bis-tris buffer solution (pH 7.0) of 50mM containing ESPT of the magnesium chloride of DS of reaction variation-per-hour 0.5mM, 2.0% of ALB, and 20mM, 0.2% of n-OTG, and 1mM, and it warmed for 5 minutes at 37 degrees C to it. 0.25ml of bis-tris buffer solution (pH 7.0) of 50mM which contains 4-AP of 0.5mM, 20micro ag [/ml] POD, and CHE and CHO of each 5u/ml in this was added, and absorbance change on the wavelength of 546nm after warming for 5 minutes at 37 degrees C was measured. It changed to serum, operation same about an HDL-cholesterol authentic sample was performed, and the HDL-cholesterol count in serum was calculated. Moreover, same operation was performed using the thing except ALB, and it was considered as contrast. On the other hand, measurement with the reaction mixture object chromatography method (Clin.Chem., such as the HPLC method, W, and Marz, 39, 2276, 1993) by a GERURO fault column was carried out about the same specimen, and it compared with the measured value. Moreover, what measured the total cholesterol value of the HDL fraction which carried out fractionation with the ultracentrifugal method beforehand with enzymatic process as a standard substance in each measuring method was used. The measured value of ten serum is shown in Table 3.
[0030]
[Table 3]
The specimen book method contrasting method HPLC method Serum 138.260.537.8 Serum 241.358.640.3 Serum 336.275.636.2 Serum 416.530.117.4 Serum 529.242.328.5 Serum 621.458.423.7 Serum 732.675.132.4 Serum 827.9 31.2 26.5 Serum 9 70.1 109.9 68.9 Serum 10 43.7 4 6.2 41.6 Unit: Mg/dl [0031] As Table 3, as compared with the contrasting method, this invention procedure has the good correlation with the HPLC method, and can measure HDL-cholesterol in serum correctly.
[0032]